PROTOCOL REVIEW

Protocols and the IACUC's approval and monitoring of protocols must be completely and thoroughly reviewed.

You (the inspector) are responsible for conducting a thorough inspection of:

- IACUC approved protocols and changes to protocols
- the IACUC's monitoring of protocol activity
- the protocol approval process

Detailed below are some aids to assist you in evaluating the IACUC. However, you must use the regulations and your professional judgment to determine if an IACUC or protocol is in compliance.

For the protocol review, you should:

1. determine the number of protocols subject to your (the inspector) review including:
   - active protocols, AND
   - inactive protocols from the past 3 years, and
   - protocols where no regulated species are present at the facility
2. if the number is small, review all of the research facility's protocols for regulated animals, OR
3. if the number is large, review a representative sample of active and inactive protocols. such as:
   - for each regulated species
   - for high profile species, such as dogs, cats, or nonhuman primates
   - for high profile procedures, such as "Specific Types of Protocols" starting on page 6.3.5
   - for different PIs
   - for each Category with animals listed on the past 3 years Annual Reports
   - protocols involving invasive procedures, e.g., skull cap placement, laparotomy, or thoracotomy
   - food and/or water restriction protocols
   - antibody production protocols
4. review all Category E protocols from the past 3 years

NOTE: The list of protocols reviewed by the IACUC may be used to determine the number of protocols and the specific protocols to be reviewed by you. Note: You may need to ask for a list of inactive protocols.

Ways to verify IACUC activities include, but are not limited to, review of:

- protocols
- protocol submission forms
- written meeting minutes
- audio meeting minutes
- correspondence
- memos/notes
- e-mail correspondence and e-mail records
- interviews with IACUC members
- Compliance Office/Officer activities, if the facility has a Compliance Office

PROTOCOL APPROVAL

Process

In assessing the protocol approval process, you should look for verification that:

- all protocols involving regulated animal use are submitted to the IACUC
- NO animal activity is started before the protocol has been properly approved
  NOTE: No IACUC member can approve a protocol or give permission for an animal activity to start before the protocol has gone through the proper approval process.
- the IACUC has a mechanism for distributing protocols and other pertinent information to IACUC members which is accessible to all members, i.e., if distributed by e-mail, all members have e-mail or an alternate method of distribution is used for members without e-mail
- all members are sent a list of protocols to be reviewed prior to the review in sufficient time to request a copy of the protocol or participate in the review

	• if the protocol was reviewed by the full IACUC: ▸ there was a quorum present ▸ approval was by a majority vote of the quorum • no IACUC member voting on the protocol had a conflicting interest • any **significant changes to protocols** were approved using the same procedures as for a protocol review • any IACUC requested additions or changes to protocols were made before final approval was given • all IACUC decisions regarding protocols, or significant changes to protocols are documented in writing and available for inspection • no official, department, or committee of the research facility overrides IACUC denials of protocols or significant changes to protocols. NOTE: Implementation of an IACUC approved protocol may be delayed or prohibited by another official, department or committee, for example, the Radiation Safety Committee if the protocol does not meet its requirements.
Notification	In assessing the protocol notification requirement, you should look for verification that: [2.31(d)(4)] • the Principal Investigator is notified in writing of the IACUC's decision on his/her protocol • the Research Facility (usually the Institutional Official or his/her designee) is notified in writing of all protocol review decisions • if protocol approval was denied, the IACUC: ▸ notified the Principal Investigator of the reason for the denial ▸ gave the Principal Investigator the opportunity to respond
Annual Review	In assessing the annual review of protocols, you should look for verification that: • all active protocols are reviewed by the IACUC or a subcommittee annually • all IACUC members are informed of the annual reviews

	• all members are given the opportunity to participate in the annual reviews • the IACUC reviews and decisions are documented in writing and available for inspection
PROTOCOL REVIEW	
General Requirements	In assessing an IACUC's review of a protocol, you should look for verification that: • the rationale for using animals is clearly stated, acceptable, and scientifically justified • the species of animal(s) to be used is clearly stated • the appropriateness of the species is adequately and scientifically justified • the number of animals to be used is clearly stated • how the approximate number of animals to be used was determined is clearly stated or shown, such as: ▸ required for statistically significant results (tests used or statisticians consulted should be included) ▸ based on scientific literature or past experience (references should be cited) ▸ based on results of pilot study ▸ required by FDA or other Federal agency (Federal code, regulation or standard, etc., must be cited) ▸ required by international testing requirements (code, regulation, standards, etc. must be cited) ▸ number of students/animal and procedures needed to learn • the proposed use of the animals is clearly and adequately detailed • the principal investigator has provided a written assurance that the proposed activity is not an unnecessary duplication of previous experiments • medical care is provided for the animals when needed • the animals' living conditions and care are adequate and appropriate • personnel conducting the research or handling the animals are

properly trained and qualified
- there is a description of how pain/distress/discomfort are minimized, if applicable
- disposition of animals at termination of study is stated, including harvesting of tissues or body parts
- the method of euthanasia is:
  - clearly stated, including drug(s) and dosages, and
  - consistent with the current *Report of the AVMA Panel on Euthanasia,* **or**
  - an alternative method justified in the protocol and approved by the IACUC
- any exemption/exception to the AWA regulations or standards is adequately justified

NOTE: Routine veterinary care, housing, euthanasia, etc., may be detailed in standard operating procedures (SOPs), but the protocol must refer specifically to that SOP(s).

Specific Types of Protocols

Painful/Distressful Procedures

When reviewing protocols involving procedures that cause more than momentary or slight pain/distress/discomfort (Protocols in Categories D & E), some areas to pay special attention to include, but are not limited to:

- the procedure is properly classified
- the principal investigator has considered alternatives to the painful/distressful procedure
- there is a detailed narrative describing the methods and sources used to determine that no alternatives to the painful/distressful procedure are available (see page 18.5.2 for electronic and non-electronic search requirements)
- measures used to alleviate the pain/distress are clearly stated, including:
  - drugs, dosages, and frequency of administration

  NOTE: A "PRN" or "as needed" frequency of administration is not acceptable unless there are detailed instructions and criteria for determining administration of the drug.
  - other methods, such as:
    - hydrotherapy
    - hot/cold packs

- measures used to relieve pain/distress are adequate, i.e., correct drug, dose, frequency, etc.
- availability of experienced personnel, especially at night and on weekends, to assess and administer pain relief
- if pain/distress relief is not to be used, there is an adequate justification (see page 18.5.3)
- the principal investigator has consulted and involved the attending veterinarian or his/her designee in the planning of the procedure and pain/distress relief
- if a paralytic is used, it is used with anesthesia
- animals experiencing severe or chronic pain/distress that cannot be relieved will be humanely euthanized
- the endpoint has been determined and identified

NOTE: If the research facility has a standard operating procedure(s) (SOP) for pain/distress relief, the protocol must reference that SOP.

Antibody Production Protocols

When reviewing protocols involving antibody production, some areas to pay special attention to include, but are not limited to:

- the principal investigator has considered alternatives for painful/distressful procedures, such as, www.nal.usda.gov/awic/pubs/antibody/overview.htm
- an alternatives search, if done, was properly conducted and reviewed for possible alternative procedures
- the justification for the number of animals to be used was appropriate, such as the amount of antibody needed and the amount which can be produced by an animal
- there is a complete description of the procedure to induce antibody production and the collection of blood/serum
- if adjuvants likely to cause more than momentary pain/distress, such as Freund's Complete, are being used, there is, at a minimum:
  - justification for its use
  - a listing of possible adverse reactions
  - adequate care of the animal if adverse reactions occur

Food and/or Water Deprivation or Restriction

When reviewing protocols involving food and/or water deprivation or restriction, some areas to pay special attention to include, but are not limited to:

- the food/water deprivation or restriction is adequately justified
- if the animals are likely to experience distress, the principal investigator has considered alternatives to the distressful procedures
- an alternatives search, if done, was properly conducted and reviewed for possible alternative procedures
- procedures used to restrict food/water are adequately described and easily understood
- procedures for selection of animals and training and monitoring the animals are described in detail
- baseline physiological data is being collected
- physiological parameters are being monitored during the study, such as:
  - body weight
  - hydration status
  - behavioral changes
  - plasma osmolality
- medical/research records are being maintained and contain information on the monitoring of the animals
- supportive care is provided to any animal suffering dehydration or stress
- if supportive care is not provided, there is an appropriate scientific justification for not doing so
- how the animals' daily food and water intake was determined
- the protocol addresses how the animal is to receive its required daily food or water intake, such as:
  - during its working sessions
  - supplementation to the amount consumed during working sessions
  - whether small amounts of food or water provided as rewards are or are not considered part of the animals' daily food or water requirement
- if the animal is not to receive its daily food or water requirement, procedures and parameters for monitoring the

animal are detailed in the protocol
- the endpoint has been determined and identified

Neuromuscular Blockers

When reviewing protocols involving the use of neuromuscular blockers (NMB), some areas to pay special attention to include, but are not limited to:

- the use of the NMB is appropriate
- the use of the NMB is adequately described in the protocol including, but not limited to:
  - name of NMB
  - dosage
  - timing of administration
  - method of anesthesia
- the NMB is being used with general anesthesia
- all personnel working with the animal and NMB are properly trained in its use and possible adverse reactions
- the animal is being properly monitored, such as:
  - heart rate and blood pressure
  - level of anesthesia. NOTE: Pain withdrawal response is **NOT** an appropriate measure of level of anesthesia.
- appropriate supportive care, such as ventilatory support, is being provided during anesthesia
- surgical and anesthesia records are being kept and contain the appropriate information
- recovery procedures are appropriate, i.e.:
  - the animal is recovered from the NMB before being allowed to recover from the anesthesia
  - recovery is being monitored

Surgical Procedures

When reviewing protocols involving surgical procedures, some areas to pay special attention to include, but are not limited to:

- the pre-procedural care and surgical preparation of the animals are clearly stated
- drugs given prior to and during the procedure, such as analgesics, tranquilizers or anesthetics, are appropriate and at the correct dosage for the species

- the surgical procedure is stated clearly and in detail
- all survival surgeries are performed using aseptic technique
- major operative survival surgeries on non-rodents are performed in a dedicated surgical facility
- no animal is being used in more that one major operative survival surgery UNLESS appropriately approved (see page 18.5.6 for requirements)
- post-surgical procedures are stated clearly and in detail, such as:
  - observation and monitoring of recovery
  - any special recovery environment requirements
- pain/discomfort relief measures are stated clearly and in detail, including but not limited to: (see page 18.5.4)
  - when drugs are to be administered
  - when or which drugs are **not** to be administered, if applicable, with an explanation
  - drug, dose, route, and frequency of administration
  - signs of pain/distress
  - contact person(s)
  - other or additional methods of pain/distress relief

NOTE: If the research facility has a standard operating procedure(s) (SOP) for surgical procedures or pain/distress relief, the protocol must reference that SOP(s).

Teaching Protocols

When reviewing teaching protocols, some areas to pay special attention to include, but are not limited to:

- the justification for the number of animals to be used was appropriate, such as the number of students per animal and procedures needed to be learned
- a consideration of alternatives was properly conducted and reviewed for possible alternative procedures, such as, the use of:
  - veterinary mannequins
  - live tissue alternatives
  - mechanical teaching devices
- there is a complete description of the procedures to be used

- the number of procedures to be performed on each animal is clearly stated, such as, injections per animal
- the personnel doing the teaching are qualified and properly trained
- if the teaching procedures cause more than momentary or slight pain or distress, proper methods are used to alleviate the pain/distress

Toxicity Studies

When reviewing protocols involving toxicity studies, some areas to pay special attention to include, but are not limited to:

- a consideration of alternatives was properly conducted and reviewed for possible alternative procedures, such as:
  - Local Lymph Node Assay
  - Up-and-Down Procedure

  (See http://iccvam.niehs.nih.gov/about/overview/htm)
- the justification for the number of animals to be used was appropriate
- if the number of animals required is set by a government agency, the specific regulation or guideline is cited in the protocol
- appropriate methods are being used to relieve any pain or distress, unless scientifically justified
- animal technicians and caretakers are properly trained in identifying problems and procedures to follow
- the end point has been determined and identified

INSPECTION PROCEDURES

Listed below are some additional aids to assist you in determining if the procedures outlined in the protocols are being followed:

- if protocol numbers are not listed on the cages, ask for the protocol numbers of random animals.
  NOTE: Animals may be held but cannot be used without being on a protocol.
- choose random protocol numbers from cage cards or animal charts/records and check in IACUC records that these protocols were approved
- ask how the research facility keeps track of the number of animals approved by the IACUC and the number of animals

used by the principal investigator, such as:
  - computer records
  - acquisition and disposition records
  - dead animal records
  - inventory cards
- ask how the facility checks the accuracy of its methods for tracking the number of animals
- ask for exemption/exceptions to the regulations or standards, then check the protocol to determine that the exemption/exception was approved
- determine if the animal care staff is familiar with the protocol procedures, especially pre- and post-painful/ distressful procedure care, such as:
  - asking the staff
  - checking the availability of protocols
  - checking the availability of standard operating procedures
  - looking in medical records
- watch the animal care staff, principal investigators or laboratory personnel handle the animals (or ask them to handle the animals)
- review medical records/investigator's logs to determine that animals with painful/distressful procedures received the proper pain/distress relieving drugs, if applicable
- observe animals for signs of unrelieved pain (see page 6.3.13)
- ask about weekend staffing, animal observation and medical care
- determine if the medical or emergency contact people's numbers are readily available, such as:
  - on bulletin boards
  - in the animal rooms
  - in medical records/charts
  - in protocols
- observe surgeries to determine that they are being conducted using aseptic technique and in dedicated surgical facilities, if required
- ask how the research facility tracks animals to ensure that they are not used for another survival surgery (unless

approved by the IACUC or APHIS), such as:

- health records
- individual animal records
- cage cards
- surgery records
- investigator's logs

- for APHIS-approved multiple major survival surgeries, verify that the stipulations in the approval letter are being met, such as:
  - approved species of animal is being used
  - surgeries performed during approved time period
  - only approved number of animals have been used
  - identification of the major operative procedure
  - only maximum number of approved survival surgeries have been conducted on the animals
  - animals have not undergone any other major survival surgery
  - all animals under the protocol are permanently identified
  - medical/surgical records accompany animals to other protocols
  - medical records include the name, dose, route, and time of administration of any medication given
  - appropriate peri-operative medication is given to the animals as directed by the attending veterinarian
  - copies of medical records are provided to any subsequent owners of the animals or any person to whom the animals are assigned
  - surgical exemption is reported on the USDA Annual Report
  - IACUC is evaluating exemption annually, including:
    - an assessment of the animals
    - effectiveness and soundness of the methods used on the animals
    - effectiveness and soundness of the procedures used on the animals
    - procedures used to minimize pain and distress
  - evaluation must be included in the IACUC reports

SPECIES-TYPICAL SIGNS OF PAIN*

SPECIES	POSSIBLE SIGNS OF PAIN**
DOGS	quiet, unwilling to move, lack of alertness, whimpering or howling, loss of appetite, increased respiration, growl or exhibit apprehension when approached, *groaning*
CATS	quiet, apprehensive facial expression, loss of appetite, crying, hissing, hiding, crouching or hunching, ungroomed appearance
GUINEA PIGS & HAMSTERS	decreased activity, piloerection, ungroomed appearance, excessive licking and scratching, rapid/shallow respiration, loss of appetite, grunting or chattering, do not try to escape when handled
RABBITS	inactive, appear apprehensive or anxious, hunched appearance, hide, squeal or cry, possible aggressive behavior with excessive scratching and licking, grinding of teeth, excessive salivation
NONHUMAN PRIMATES	huddling or crouching in corner, stops eating/drinking, sad expression, moaning, screaming, stops grooming, clenching of teeth
CATTLE, SHEEP, GOATS	dull, depressed appearance, heads bowed, lack of alertness, loss of appetite, rapid/shallow breathing, rigid posture, vocalization, *droopy ears, rough hair coat, hunched appearance*
PIGS	changes in social behavior, gait and posture, *excessive* squealing when handled, unwilling to move, hiding

**These are possible signs of pain and do not necessarily mean the animal is in pain. A lack of these signs also does not mean that the animal is not in pain.
Italics - added by Manual Team

*excerpted from: National Research Council: Recognition and Alleviation of Pain and Distress in Laboratory Animals, Washington, D.C., National Academy Press, 1992.